Reconstruction Algorithms for Next-Generation Imaging: Multi-Tiered Iterative Phasing for Fluctuation X-ray Scattering and Single-Particle Diffraction

Jeffrey J. Donatelli

Mathematics Department, Lawrence Berkeley National Laboratory
Center for Advanced Mathematics for Energy Research Applications (CAMERA)
Emerging Imaging Techniques Enabled by XFELs

Fluctuation X-ray Scattering (FXS)

Single-Particle Diffraction (SPD)
Outline of Talk

1) Fluctuation X-ray Scattering (FXS)
   i) Description of experiment
   ii) Mathematical formulation of the FXS reconstruction problem
   iii) Multi-tiered iterative phasing (M-TIP) for FXS reconstruction
   iv) Experimental challenges
   v) Reconstructions from experimental data

2) Single-Particle Diffraction (SPD)
   i) Description of experiment
   ii) Single-particle M-TIP for SPD reconstruction
   iii) Results
Fluctuation X-ray Scattering (FXS)

▶ Several X-ray diffraction images are collected from particles in solution using X-ray exposures below rotational diffusion times

▶ Multiple particles per shot

▶ Images contain angular fluctuations:

Angular correlations are computed from each image \( J^{(n)} \) and then averaged:

\[
C(q, q', \Delta \phi) = \left\langle \frac{1}{2\pi} \int_{0}^{2\pi} J^{(n)}(q, \phi + \Delta \phi) J^{(n)}(q', \phi) d\phi \right \rangle^{n}
\]

▶ Medium to high resolution structure can be determined from the correlation data


**SAXS vs. FXS**

### Small Angle X-ray Scattering (SAXS)

Particles rotate during imaging:

Full rotational integration:

\[
\text{Image} = \int_{SO(3)} I_R dR
\]

Images are angularly isotropic:

Can only extract angular average: \((J(q, \phi) = \text{Image})\)

\[
\text{SAXS}(q) = \int_{0}^{2\pi} J(q, \phi) d\phi
\]

### Fluctuation X-ray Scattering (FXS)

Particles are frozen in place during imaging:

Finite rotational averaging:

\[
\text{Image} = \sum_{j=1}^{M} I_{R,j}
\]

Images contain angular fluctuations:

Can extract angular correlations: \((J(q, \phi) = \text{Image})\)

\[
C(q, q', \Delta \phi) = \int_{0}^{2\pi} J(q, \phi) J(q', \phi + \Delta \phi) d\phi
\]
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Coordinate Notation:

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<td>( \mathbf{q} )</td>
<td>((q, \theta, \phi))</td>
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Electron Density: \( \rho(\mathbf{r}) \) (Gray isosurface)

Structure Factors:

\[
\hat{\rho}(\mathbf{q}) = \int_{\mathbb{R}^3} \rho(\mathbf{r}) e^{-2\pi i \mathbf{q} \cdot \mathbf{r}} d\mathbf{r}
\]

Intensity Function: Diffraction measures information about the intensity function

\[
I(\mathbf{q}) = |\hat{\rho}(\mathbf{q})|^2
\]

Goal: Determine information about the electron density \( \rho \) from a set of diffraction images
Spherical Harmonics - “Fourier Series on a Sphere”

Spherical Harmonic Expansion:

\[ I(q, \theta, \phi) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} I_{lm}(q) Y_{lm}(\theta, \phi) \]

Spherical Harmonic Transform:

\[ I_{lm}(q) = \int_{0}^{2\pi} \int_{0}^{\pi} I(q, \theta, \phi) Y_{lm}^*(\theta, \phi) \sin \theta d\theta d\phi \]

Vector Format:

\[ I_l(q) = \left[ I_{l(-l)}(q) \quad I_{l(1-l)}(q) \quad \ldots \quad I_{l0}(q) \quad \ldots \quad I_{l(l-1)}(q) \quad I_{ll}(q) \right]^T \]

Matrix Format:

\[ I_l = \begin{bmatrix} I_{l(-l)}(q_1) & I_{l(1-l)}(q_1) & \ldots & I_{l0}(q_1) & \ldots & I_{l(l-1)}(q_1) & I_{ll}(q_1) \\ I_{l(-l)}(q_2) & I_{l(1-l)}(q_2) & \ldots & I_{l0}(q_2) & \ldots & I_{l(l-1)}(q_2) & I_{ll}(q_2) \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots & \vdots \\ I_{l(-l)}(q_N) & I_{l(1-l)}(q_N) & \ldots & I_{l0}(q_N) & \ldots & I_{l(l-1)}(q_N) & I_{ll}(q_N) \end{bmatrix} \]
Relation between FXS and the 3D Intensity Function

**Average Correlation Function:** (Images: $J^{(1)}, \ldots, J^{(N_{dp})}$)

$$C(q, q', \Delta \phi) = \frac{1}{2\pi} \int_0^{2\pi} J^{(n)}(q, \phi + \Delta \phi) J^{(n)}(q', \phi) d\phi$$

**Legendre Polynomial Expansion:** ($N_{dp} \to \infty$, uniformly random orientations)

$$C(q, q', \Delta \phi) = \frac{1}{4\pi} \sum_{l=0}^{\infty} P_l \left( \cos \theta(q) \cos \theta(q') + \sin \theta(q) \sin \theta(q') \cos \Delta \phi \right) B_l(q, q'),$$

where $\theta(q) = \arccos(q\lambda/2)$, and, up to a set of scaling factors

$$B_l(q, q') = \sum_{m=-l}^{l} I_{lm}(q) I_{lm}^*(q')$$

Features:

- Uncorrelated noise effects (e.g. shot noise) vanish when averaging the correlations over a sufficient number of images (apart from a sharp “noise peak” at $q = q', \Delta \phi = 0$)

- For $M$ particles per shot, $B_0 \sim M^2$ and $B_l \sim M$ for $l > 0$
In order to determine structure from correlation data, two tiers of phase problems must be solved:

1) **Classical Phase Problem** \((I(q) = |\hat{\rho}(q)|^2)\)

   Scalar Phase Problem
   \[
   \hat{\rho}(q) = \sqrt{I(q)} e^{i\phi(q)}
   \]

   The complex phases \(\phi(q)\) need to be determined in order to recover \(\rho\).

2) **Hyperphase Problem**

   a) **Autocorrelation Data**: \((B_l(q) := B_l(q, q), B_l(q) = ||I_l(q)||^2)\)

   Vector Phase Problem
   \[
   I_l(q) = \sqrt{B_l(q)} u_l(q), \text{ where } u_l^*(q)u_l(q) = 1
   \]

   The phase vectors \(u_l(q)\) need to be determined in order to recover \(I\).

   b) **Cross-Correlation Data**: \((B_l(q, q'), B_l = I_lI_l^*)\)

   Rank \(2l + 1\) Eigenvalue Decomposition: \(B_l = V_l\Lambda_l V_l^* = (V_l\sqrt{\Lambda_l})(V_l\sqrt{\Lambda_l})^*\)

   Matrix Phase Problem
   \[
   I_l = V_l\sqrt{\Lambda_l} U_l, \text{ where } U_l^*U_l = I_{2l+1}
   \]

   The phase matrices \(U_l\) need to be determined in order to recover \(I\).
FXS Reconstruction Problem: Determine the electron density $\rho$ given the $B_l(q, q')$ quantities along with additional constraints.

- Complex phases $\phi(q)$ need to be recovered (classical phase problem)
- Phase vectors $u_l(q)$ or phase matrices $U_l$ need to be recovered (hyper-phase problem)
- Additional constraints on the solution are needed to make the problem well-posed (e.g. constraints on size, positivity, symmetry, density statistics)
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1) Develop a set of Bregman projection operators, each of which projects a model estimate to the closest object that satisfies a given constraint.

**Model Constraints:**
- Consistency with the $B_l$ data
- Compact support
- Nonnegativity
- Symmetry
- Similarity to another structure
- Statistical properties

2) Starting with a random initial model, iteratively apply projections to enforce constraints until the projected models are consistent with all constraints and data.
Real-Space Projectors

- **Support Projector:** To enforce a support region $S$

  $$(P_S \rho)(\mathbf{r}) = \begin{cases} \rho(\mathbf{r}), & \text{if } \mathbf{r} \in S, \\ 0, & \text{otherwise.} \end{cases}$$

- **Nonnegativity:** $P_+ \rho = \max(\rho, 0)$

- **Upper Bound:** $P_\tau \rho = \min(\rho, \tau)$

- **Combinations:** $P_{S+}, P_{S\tau}, P_{S+\tau}$

- **Symmetry Projector:** To enforce symmetry given by a point group $G \subseteq O(3)$

  $$(P_G \rho)(\mathbf{r}) = \frac{1}{|G|} \sum_{\mathcal{O} \in G} \rho(\mathcal{O}\mathbf{r}).$$

  $$P_G(\text{Density}) = \text{Support}$$
Fourier-Space Projectors

- **Magnitude Projector:** Projects density \( \rho \) to satisfy \( I = |\hat{\rho}|^2 \)

\[
(P_M(I)\rho)(q) = \sqrt{I(q)} \frac{\hat{\rho}(q)}{|\hat{\rho}(q)|}
\]

- **Autocorrelation Projector:** Projects intensity \( I \) to satisfy \( B_l(q) = ||I_l(q)||^2 \)

\[
(P_A I)_{l}(q) = \sqrt{B_l(q)} \frac{I_l(q)}{||I_l(q)||}
\]

- **Cross-Correlation Projector:** Projects intensity \( I \) to satisfy \( B_l = I_l I_l^* \) \( (B_l = V_l \Lambda_l V_l^*) \)

\[
(P_C I)_{l} = V_l \sqrt{\Lambda_l} U_l, \quad U_l = \arg \min_{U_l \in U(2l+1)} ||D(I_l - V_l \sqrt{\Lambda_l} U_l)||_F
\]
Multi-Tiered Iterative Phasing (M-TIP)

Real-Space Projector:
\[ P_{S*} \]

Fourier-Space Operator:
\[ F\rho = P_M(P_A|\hat{\rho}|^2)\rho \text{ or } P_M(P_C|\hat{\rho}|^2)\rho. \]

Error Reducing (ER) Scheme:

\[ \rho^{(n+1)}(r) = (P_{S*}F\rho^{(n)})(r). \]

Hybrid Input-Output (HIO) Scheme:

\[ \rho^{(n+1)}(r) = \begin{cases} (F\rho^{(n)})(r), & \text{if } (P_{S*}F\rho^{(n)})(r) = 0, \\ \rho^{(n)}(r) - \beta(P_{S*}F\rho^{(n)})(r), & \text{otherwise}, \end{cases} \]

where \( P^c \rho = \rho - P\rho \) and \( 0 < \beta \leq 1 \).

Reconstructions of pLGIC from Simulated FXS Data

Original

Reconstruction from FXS Cross-Correlation data
$B_l(q, q'), l \leq 20$

Reconstruction from FXS Cross-Correlation data
$B_l(q, q'), l \leq 20$
5-fold symmetry constraint
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Systematic Issues in Experimental FXS Data

How to remove systematic issues and noise:

- Correct for issues directly in each image
- Remove effects by subtracting out correlations of average of all images and blank shots:
  \[ C_{\text{filtered}} = C_{\text{hits}} - C_{\text{averageimage}} - C_{\text{misses}} \]
- Model remaining issues during the reconstruction process (Noise projectors)
- Filtering via sparse representations
Instead of fitting directly to the noisy observed data \( D^{\text{obs}} \), we fit to a noise-filtered version which is updated during each iteration via

\[
P_N D^{\text{mod}} = D^{\text{mod}} + \Delta D,
\]

where \( D^{\text{mod}} \) is simulated by solving the forward problem with the current model and \( \Delta D \) solves

\[
\min ||\Delta D|| \quad \text{subject to} \quad \frac{1}{N_{\text{data}}} \sum_i \left( \frac{(D^{\text{mod}} + \Delta D)_i - D^{\text{obs}}_i}{\sigma_i^2} \right)^2 < 1.
\]

**Example:** 2D Phase Retrieval from Noisy Intensity Measurements

Original Model

Diffraction Pattern with Gaussian Noise

Clean Diffraction Pattern

Iterative Phasing without Noise Projector

Iterative Phasing with Noise Projector
Variances $\sigma_i^2$ can be estimated via symmetry violation about $\Delta \phi = \pi/2, \pi, 3\pi/2$!

M-TIP can be modified to model noise by applying the noise projector in an additional tier.

\[
\begin{align*}
\rho(\mathbf{r}) & \rightarrow \hat{\rho}(\mathbf{q}) & | \cdot |^2 & \rightarrow I(\mathbf{q}) & \text{SHT} & I_{\text{im}}(\mathbf{q}) & \rightarrow B_i(q, q') & IALT & C(q, q', \Delta \phi) \\
\text{Real-Space Constraints} & \quad \text{FT} & P_S, P_{S+}, & P_{S+\tau}, & P_G & \quad \text{Phases} & \text{Hyperphases} & \text{FXS Data} & C_{\text{obs}}(q, q', \Delta \phi) \\
\rho_{\text{new}}(\mathbf{r}) & \rightarrow \hat{\rho}_{\text{new}}(\mathbf{q}) & \text{IFT} & I_{\text{new}}(\mathbf{q}) & \text{ISHT} & I_{\text{new}}(\mathbf{q}) & \text{P}_A, \text{P}_C & \text{ALT} & C_{\text{new}}(q, q', \Delta \phi) \\
\end{align*}
\]
The correlation function can be expressed in terms of a small number of basis elements:

**Low-Rank Legendre Decomposition:** \((l_{\text{max}} \approx \pi q_{\text{max}} D, \, D = \text{particle size})\)

\[
C(q, q', \Delta \phi) = \sum_{l=0}^{l_{\text{max}}} P_l(\cos \theta(q) \cos \theta(q') + \sin \theta(q) \sin \theta(q') \cos \Delta \phi) B_l(q, q')
\]

**Low-Rank Spherical-Bessel Decomposition:** \((K_l \approx q_{\text{max}} D - l/\pi)\)

\[
B_l(q, q') = \sum_{k_1=1}^{K_l} \sum_{k_2=1}^{K_l} b_{lk_1}(q) b_{lk_2}(q') c_l(k_1, k_2),
\]

where

\[
b_{lk}(q) = \frac{u_{l,k}}{u_{l,k}^2 - (2\pi q D)^2} j_{l+1}(u_{l,k}) j_l(2\pi q D)
\]

**Low-Rank Eigenvalue Decomposition** \((2l+1 \text{ nonzero eigenvalues})\)

\[
c_l(k_1, k_2) = \sum_{i=1}^{2l+1} \lambda_{li} v_{li}(k_1) v_{li}(k_2), \quad \lambda_{l1}, \ldots, \lambda_{l2l+1} \geq 0
\]

Enforcing this low-rank expansion allows us to drastically filter the correlation data!

- Can be performed by combining the noise projector, regularized linear inversion, and principal component analysis
Correlation Filtering via Low-Rank Decompositions - Example

**Simulated Data Size**
- \( N_q = 320 \), \( N_{\Delta \phi} = 400 \)
- \( N_q^2 \times N_{\Delta \phi} = 4 \times 10^7 \) total measurements
- \( 4 \times 10^2 \) degrees of freedom
- \( 10^5 \) data redundancy

\[ \text{Noisy SNR} = \frac{1}{500} \]
\[ \text{Filtered SNR} = 200 \ (10^5 \text{ improvement!}) \]
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   v) **Reconstructions from experimental data**

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Experimental FXS Data from CroV and PBCV (Back Panel Only)

**CroV Experimental Data**
- 1-5 particles per shot
- 30K images
- 50 nm resolution at detector edge

**PBCV Experimental Data**
- 50-100 particles per shot
- 60K images
- 50 nm resolution at detector edge

Credit: Proposal Team - Kerfeld, Schlichting, Ourmazd, Fromme & Zwart teams
Data Processing and Structure Solution - Donatelli, Malmerberg & Zwart
Reconstructions from Experimental Autocorrelation Data

**CroV Reconstruction** ($l \leq 12$, no symmetry enforced)

Isodensity surface

2D slice

$B_l(q)$ fits

**PBCV Reconstruction** ($l = 0, 6$, icosahedral symmetry enforced)

Isodensity surface

2D slice

$B_l(q)$ fits
Reconstruction of PBCV from Experimental FXS Data (With Front Panel, 12 nm data)

EM Data Bank Structure

Reconstruction from Experimental Autocorrelation Data
(icosahedral symmetry enforced)
Experimental Single-Particle Diffraction Data from RDV and PR772

**RDV Experimental Data**
- 1 particle per shot
- 332 images
- 12 nm resolution at detector edge

**PR772 Experimental Data**
- 1 particle per shot
- 566 images
- 12 nm resolution at detector edge

Credit: Proposal Team - Single Particle Initiative Data Processing and Structure Solution - Kurta, Donatelli, Yoon, & Zwart
Reconstructions from Experimental Cross-Correlation Data (No symmetry enforced)

**RDV**

Resolution: FSC - 13.5 nm, PRTF - 17.7 nm

**PR772**

Resolution: FSC - 12.6 nm, PRTF - 16.9 nm

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Diffraction patterns are collected from individual molecules, i.e., one particle per shot.

Each particle is delivered to the beam at random and unknown orientations through a liquid droplet or aerosol delivery system.
Single-Particle Diffraction Images:
Image $J^{(k)}$ samples $I$ along a spherical slice rotated according to the image orientation $R_k$:

$$J^{(k)}(q, \phi) = I^{(R_k)}(q, \theta(q), \phi),$$

where $\theta(q) = \arccos(q\lambda/2)$.

Challenges:
1) Orientation Problem: Determine the orientation $R_k$ of each image $J^{(k)}$.
2) Intensity Reconstruction: Extract the 3D intensity function $I$ from the set of images.
3) Classical Phase Problem: Reconstruct the electron density $\rho$ from the intensity function $I$. 
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M-TIP for Single-Particle Diffraction (SPD)

**SPD Operators:**

1) **Orientation Matching:** Aligns each image to the current 3D intensity model

2) **Intensity Synthesis:** Projects the current 3D intensity model to be as consistent as possible with the set of 2D images at their estimated orientations

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Original Model of pRb: (5.5 Å)

Reconstructions from simulated clean SPD data:

- 24 images, 5.8 Å
- 12 images, 6.3 Å
- 6 images, 7.5 Å
Reconstruction from Mixed-State SPD Data with Single-Particle M-TIP

Sialic acid binding protein (SiaP) in its open and closed states:

Original Models (5.5 Å):

Reconstructions from simulated mixed-state SPD data: (48 images, 24 from each state)
Reconstruction from Noisy SPD Data with SP M-TIP + Noise Projector

Images with Simulated Shot Noise (5.5 Å, 8000 photons/image, 0.25 photons/Shannon-angle at edge)

Clean Image

Noisy Image

192 images 6.4 Å

384 images (192 images per state) 6.3 Å 6.8 Å
Single-Image Reconstructions of RDV and PR772 with SP M-TIP

RDV

SP M-TIP with Icosahedral constraint

PR772

SP M-TIP with Icosahedral constraint
Emerging techniques enabled by XFELs:

1) Fluctuation X-ray Scattering (FXS) - Measure angular correlations from the diffraction patterns of particles in solution
   - Allows for multiple particles per shot (100% hit rates)
   - Does not require knowledge of particle orientations
   - Robust to uncorrelated noise

2) Single Particle Diffraction (SDP) - Collect diffraction images from single molecules
   - Reveals structural information about individual molecules
   - More information per image compared to FXS

Multi-tiered iterative phasing (M-TIP):
   - General iterative reconstruction framework for simultaneously determining all degrees of freedom
   - Able to boost the effective information content of the system via enforcement of real-space constraints during each iteration
   - Can determine 3D structure from FXS without enforcing symmetry
   - Can determine 3D structure from SPD from a sparse set of images

Future Work:
   - Model experimental error and uncertainty as part of the M-TIP reconstruction process
   - Scale up single-particle M-TIP code to use more RDV & PR772 data and model more heterogeneity
   - Extend M-TIP to other experiments
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  - Fromme team (ASU)
  - Ourmazd team (UWM)
  - Zwart team (LBNL)
- Single Particle Initiative
Computation of Integral Transforms

Polar Grid:
- radial nodes: \( r_n = \frac{n}{N}, q_n = \frac{Qn}{N} \)
- inclination angle nodes: \( \theta_{l'} = \arccos(x_{l'}) \), where \( x_{l'} \) are Gaussian quadrature nodes with weights \( w_{l'} \)
- azimuth angle nodes: \( \phi_{m'} = \frac{2\pi m'}{M} \)

Discrete Spherical Harmonic Transform: \((\text{FFT} + \text{Gaussian quadrature})\)

\[
\rho_{lm}(r_n) = \frac{1}{M} \sum_{l'} \sum_{m'} \rho(r_n, \theta_{l'}, \phi_{m'}) Y_{l'}^{m*}(\theta_{l'}, \phi_{m'}) w_{l'}.
\]

Polar Fourier Transform: Computed by applying the spherical Hankel transform

\[
\hat{\rho}_{lm}(q_n) = 4\pi (-i)^l \int_0^\infty \rho_{lm}(r) j_l(2\pi q_n r) r^2 dr,
\]
using a sine/cosine series approximation and then evaluating the spherical harmonic series

\[
\rho(q_n, \theta_{l'}, \phi_{m'}) = \sum_l \sum_m \hat{\rho}_{lm}(q_n) Y_l^{m*}(\theta_{l'}, \phi_{m'}).
\]
Complication - FXS theory assumes that the scattering from multiple particles add incoherently, but XFELs have a fully coherent beam!

- Assume $M > 1$ particles per shot
- $A_j(x)$ - complex scattering from the $j$-th particle at pixel $x$

**Incoherent Sum:**

$$\text{Image}_{\text{incoh}}(x) = \sum_{j=1}^{M} |A_j(x)|^2$$

**Coherent Sum:**

$$\text{Image}_{\text{coh}}(x) = \left| \sum_{j=1}^{M} A_j(x) \right|^2$$
Interparticle Coherence Effects (cont’d)

Relation between correlations from a coherent and incoherent sum: \((d = \text{beam} \times \text{sample density})\)

\[
C_{\text{coh}}(q_1, q_2) = C_{\text{incoh}}(q_1, q_2) \\
+ a_1 |\hat{d}(q_2)|^2 + a_2 |\hat{d}(q_1)|^2 + a_3 |\hat{d}(q_1 - q_2)|^2 + a_4 |\hat{d}(q_1 + q_2)|^2 \\
+ a_5 \Re[\hat{d}(q_1 - q_2)\hat{d}(-q_1)\hat{d}(q_2)] + a_6 \Re[\hat{d}(q_1 + q_2)\hat{d}(-q_1)\hat{d}(-q_2)] \\
+ a_7 |\hat{d}(q_1)|^2 |\hat{d}(q_2)|^2
\]

For a sufficiently large and smeared-out sample volume and beam, \(\hat{d} \approx \delta\) (Dirac Delta function):

\[
C_{\text{coh}}(q_1, q_2) = C_{\text{incoh}}(q_1, q_2) + b_1 \delta(q_1) + b_2 \delta(q_2) + b_3 \delta(q_1 - q_2) + b_4 \delta(q_1 + q_2)
\]

In polar coordinates:

\[
C_{\text{coh}}(q, q', \Delta \phi) = C_{\text{incoh}}(q, q', \Delta \phi) + c_1 \delta(q) + c_2 \delta(q') + c_3 \delta(q - q') \delta(\Delta \phi) + c_4 \delta(q - q') \delta(\Delta \phi - \pi)
\]

**Coherence effects are concentrated in peaks at \(q\) or \(q' = 0\) and \(q = q'\) for \(\Delta \phi = 0\) or \(\pi\), which can be masked out!**
Comparison of FXS Reconstruction Methods

**Serial Approach:** Solve the hyperphase problem and then solve the classical phase problem

- In general, insufficient number of constraints on the intensity to determine the hyperphases
- Can be performed for icosahedral, helical, or cylindrical particle symmetry [1-5]

**Black-box Optimization:** Find density that is consistent with data via black-box optimization [6]

- Leverages density constraints to determine hyperphases (No symmetry requirement)
- Unable to exploit structure of the problem
- Poor convergence properties - can require week of computing hours to determine low-resolution structure

**Multi-Tiered Iterative Phasing:** Iteratively update estimates for the density, scalar phases, and hyperphases via projections [7]

- Leverages density constraints to determine hyperphases (No symmetry requirement)
- Fully exploits structure of the problem to increase accuracy and accelerate convergence
- Fast - medium to high resolution reconstructions require 5-30 minutes on a single CPU core

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Comparison of SPD Reconstruction Algorithms

Current Approaches to SPD Reconstruction:

- Manifold embedding [1-6]
- Common curve analysis [7-10]
- Expectation maximization (EMC) [11-13]

The above approaches are serial in nature - they solve the problem in two separate steps:

1) Orientation determination and intensity reconstruction
2) Phasing - to determine the electron density from the reconstructed intensity function

Single-Particle Multi-Tiered Iterative Phasing (M-TIP) [14]

- Iteratively updates estimates for the density, phases, orientations, and intensity
- Can leverage real-space density constraints to help determine orientations and boost information content of the system (Reduces number of required images)